

# Reverse phase HPLC of class I HLA eluted peptide ligands

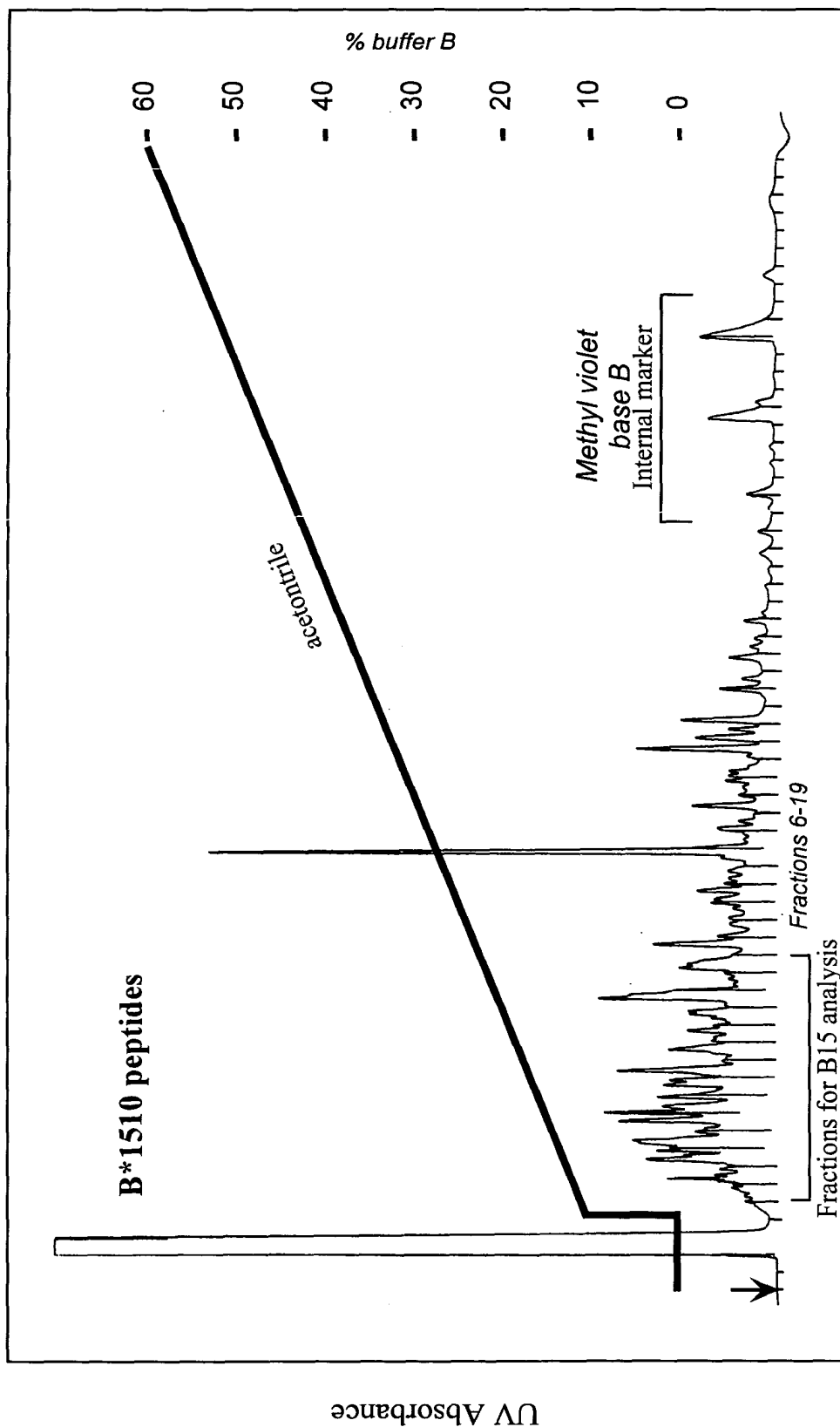


Fig. 1

Ion maps of peptides eluted from various B15 class I sHLA molecules. Mapping was accomplished with a nano-spray needle and an ESI mass spectrometer. The figure shows that the same ion peak is present in 3 of 4 B15 class I.

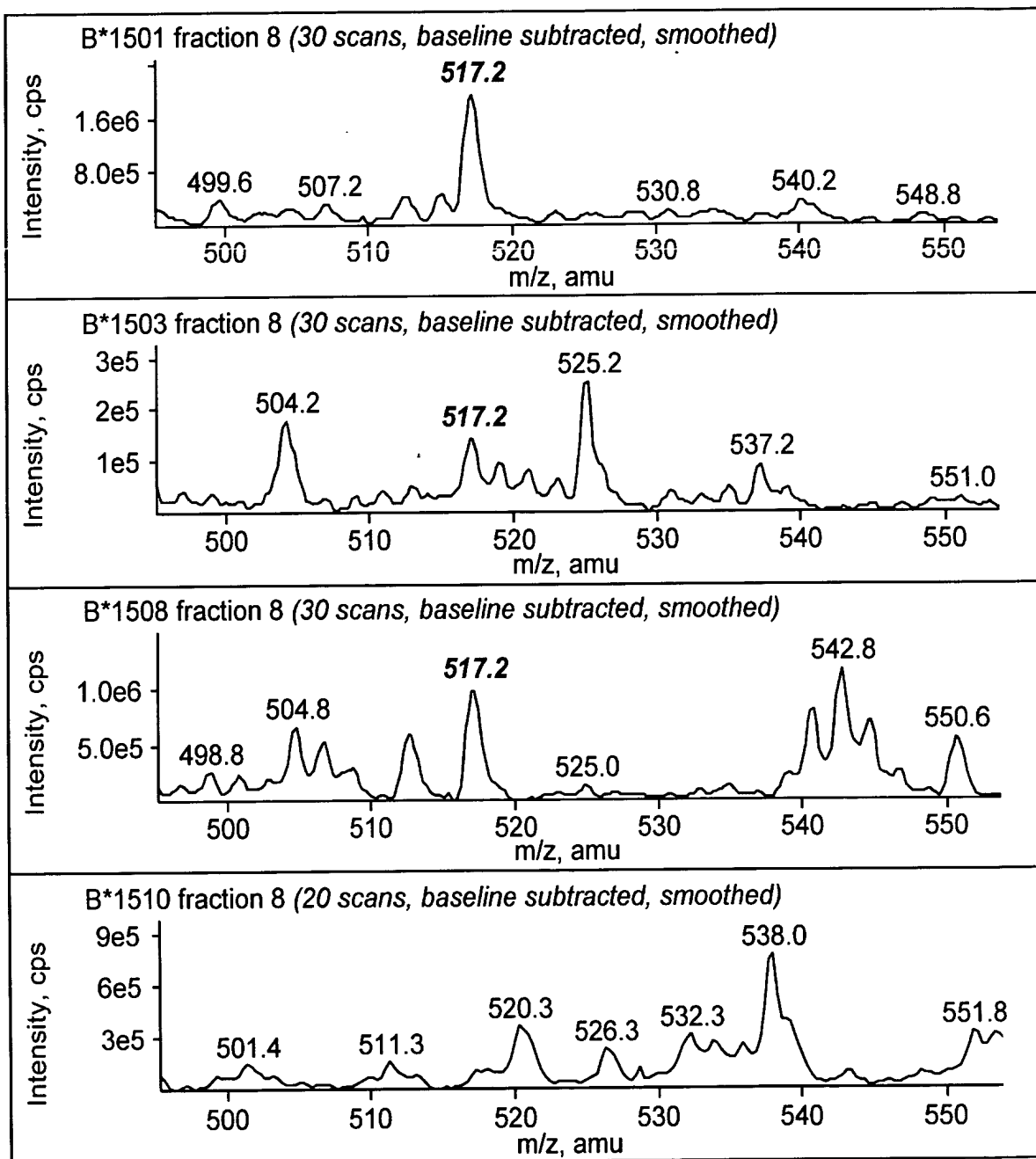


Fig. 2

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MS/MS fragmentation-sequencing of ion 517.2 from the various B15 class I sHLA molecules. This data was accomplished by completing a second nanospray of the peptides in fraction 8 from the HPLC. This demonstrates how ions can be MS ion mapped and subsequently MS/MS sequenced. There is sufficient peptide present to do multiple MS/MS fragmentation runs. There is also sufficient peptide present to facilitate a submotif on fraction 8 or further separation in the event that two peptides had mapped at 517.2 in the ion map.

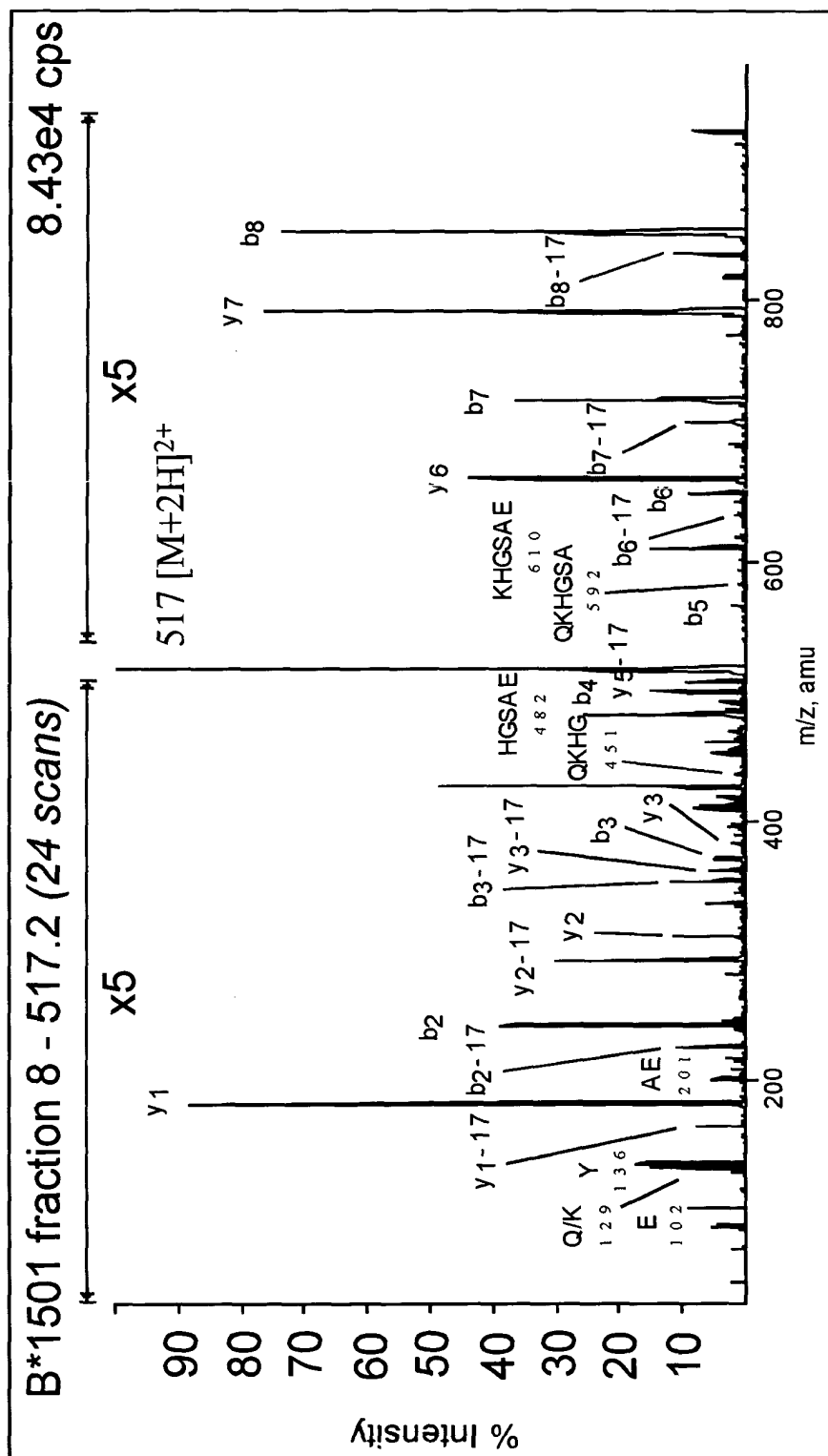


Fig. 3 1 of 3

Serial No. 10/082,034      Dkt. No. 6680.040  
 Title: SOLUBLE HLA LIGAND DATABASE UTILIZING  
 PREDICTIVE ALGORITHMS AND METHODS OF MAKING  
 AND USING SAME  
 Applicant: William Hildebrand      Group No.: 2171  
 Filed: 02/21/2002      Examiner: Unknown  
 Atty: Douglas J. Sorocco      Tel: (405) 478-5344

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2092910-HE02800F

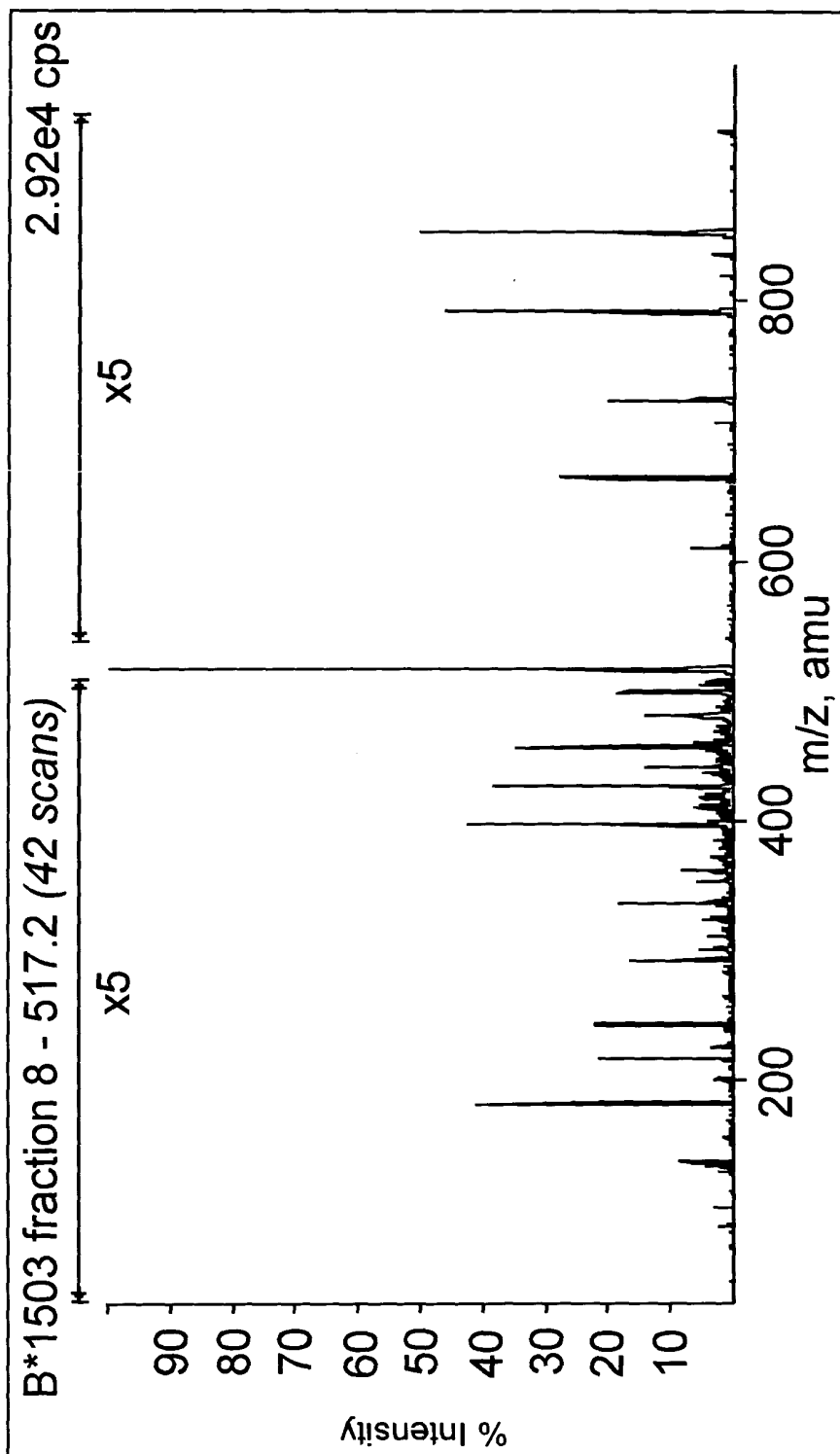


Fig. 3 2 of 3

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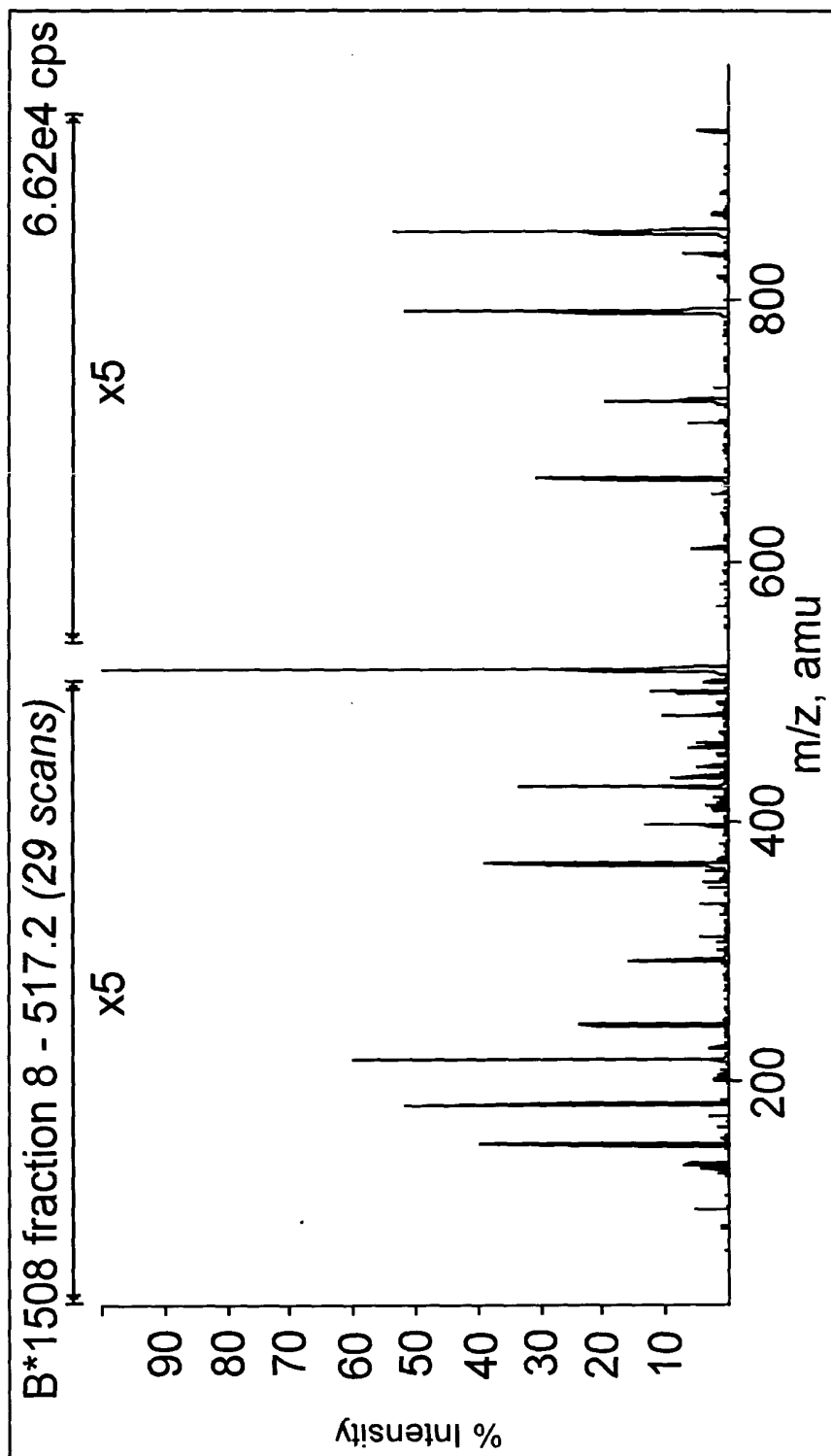
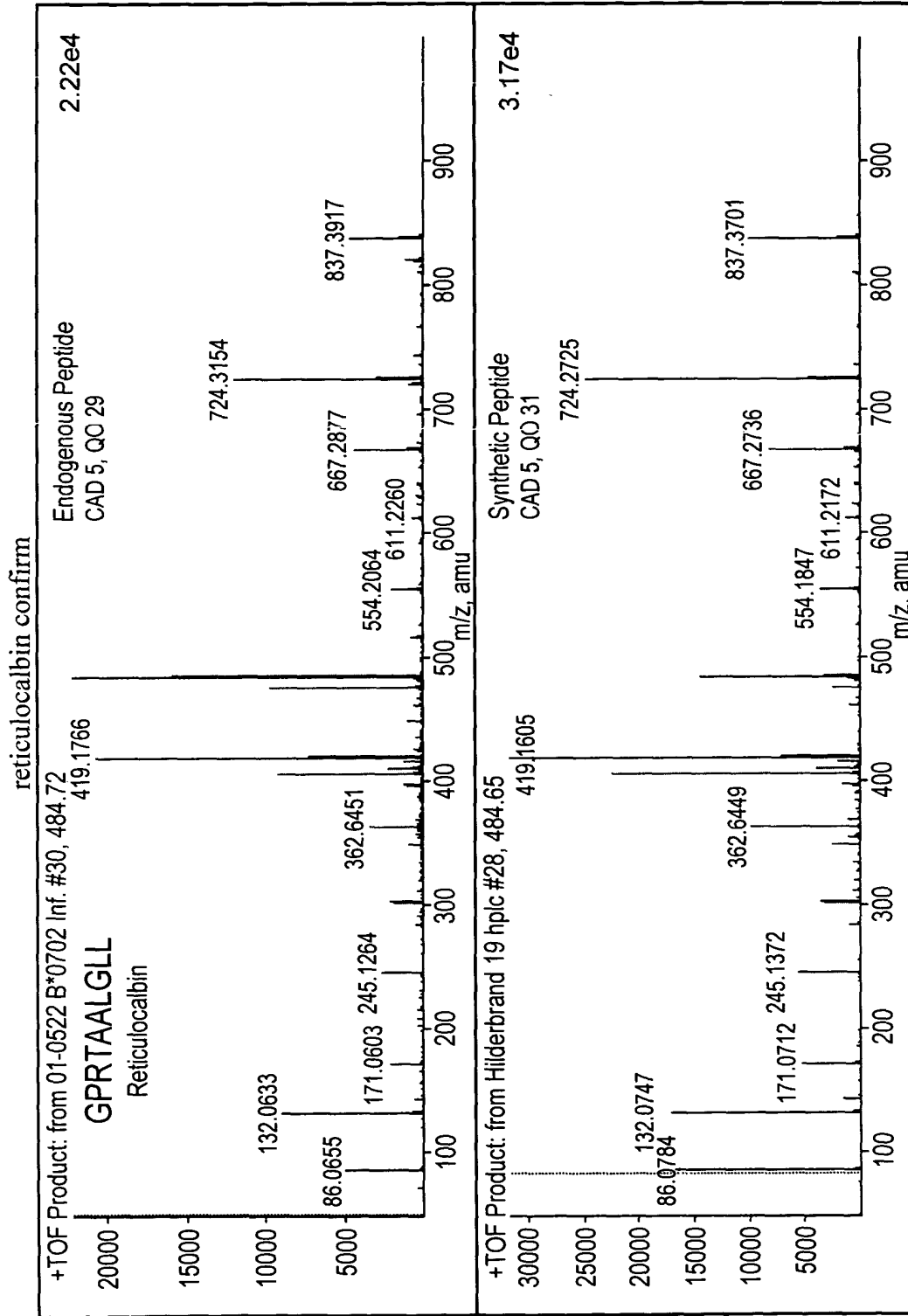


Fig. 3 3 of 3



sHLA B\*0702 was secreted from HIV infected and uninfected cells. The ion maps of the peptides eluted from sHLA B\*0702 in infected and uninfected cells were compared. Ion 484.72 was unique to the HIV infected cells. Ion 484.72 was subjected to MS/MS fragmentation-sequencing. We called GPRTAALGLL as the sequence of the ligand. We synthesized this peptide and found that it generated the same MS/MS fragmentation pattern as the ligand from HIV infected cells. This MS/MS data on a synthetic ligand matches our experimental data and validates the accuracy of our sequence.

Fig. 4

209230 "HEADBOOT"

Serial No. 10/082,034 Dkt. No. 6680.040  
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PREDICTIVE ALGORITHMS AND METHODS OF MAKING  
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Applicant: William Hildebrand Group No.: 2171  
Filed: 02/21/2002 Examiner: Unknown  
Atty: Douglas J. Sorocco Tel: (405) 478-5344

B\*1508

1	2	3	4	5	6	7	8	9
-	P	N	-	-	-	-	-	Y
-	A	F	K	R	H	Y	I	
-	-	-	E	H	R	-	-	F
-	-	-	D	G	S			

B\*1501

1	2	3	4	5	6	7	8	9
-	Q	K	-	-	-	-	-	Y
-	F	N	R	Y				
-	M	P	P	G	-	-	-	F
-	L	H	D	I				
-	V	A	G	E				

B\*1510

1	2	3	4	5	6	7	8	9
-	H	Y	P	-	-	-	-	L
-	A	I	K	S	F	L	V	T
-	-	-	G	R	R	I	M	-
-	-	-	D	D	V	L		
-	-	-	E	K				
-	-	-	M	E				
-	-	-	N					

Fig. 5

--WDRHTXF  
-----YT  
XAZYEHY  
NGXAMHWTY  
VPCGZSY  
TAZXHRGY  
NQZHGSAEY  
TG-----AY  
-P--GZDEV  
NGYDGNAGY  
TPXGEPYZSY  
FVSNHAY  
SOFGGGSOY  
SOFDHVTY  
XAN--VT  
VDX---Y  
CPLSCFT  
FLZAMZSTY  
TVXDSZTHY  
DPSGTZSGF  
-A-PHPMGY  
AQTVGIGEY  
-----SVY  
TGNCSTGTGY  
AQNVPSTY  
TP--ARAFT  
SPGAETRAY  
YX---RTF  
YXG---GAF  
-P---PSSGY  
TP---GRMY  
PMFDZZVY  
AQEHGCAAZF  
-M---GVHDI  
YVS--RNZY  
XLFH--ET

AQYAAAGESFY  
TPHTZHDY  
YM---FMY  
MVGXXPAT  
DPHYVSGHZF  
VACV---Y  
PLA-N-HTY  
VAPITTY  
XAXYRMY  
PXAMXYTY  
-P-MPGXAY  
--TZNAY  
MAAMVGAVY  
LPHQPLATY  
FVTXNXY  
GPZVMZHG  
FOARXTEY  
AAAXV---VTY  
XPEMGZFSY  
YV--VR-VF  
AAPVGAXESY  
YVA--PAF  
VGY--AHPGF  
-----STY  
SPTYTHAVAF  
MPA--MVMAF  
XA---SYTY  
VGYVDDTQF  
ZATNSVTSTY  
YATAGEMMAF  
TARXSVVEY  
MPAADYEVAF  
AAFCG---XV  
SPNEDXMZVF  
VAATAGAVF  
XLFH--ET

ILGPPGGSVY  
XLGDVNMV  
AP-----XVSY  
YMSGXYGTF  
---AVVAZSY  
MPAGYNNVY  
XPVVPAAZTY  
YMDPSGVSY

--WDRHTXF  
-----YT  
ALGA--RGY  
XS---VEY  
AQFASGAGZ  
-G--CDY  
---ZARGY  
ALNGRVTMY  
DPHAPPZY  
XAZVZMTAY  
NQZHGSAEY  
FGXACXATSY  
APMARGZY  
GOZZAVDF  
TPXGEPYZSY  
GOHASVXSY  
FVSNHAY  
-----SYGORKGAGSVF  
NPPAZZPN  
-Q-DPPFDMZY  
XQ--AGGZY  
SOFGGGSOY  
SOFDHVTY  
--AAHVPPGY  
FMDVGAPT  
XAN--VT  
AQM--SEY  
CPLSCFT  
FLZAMZSTY  
TVXDSZTHY  
AQAAFPAGY  
ALW--PZF  
VPHZNAY  
-----GHGGY  
VVATZNZZX

FLZAMGSTY  
GOYVZPTY  
GMFDPZTF  
XAVGHSGGT  
-----PTY  
EPAMVZCF  
XAHTCEPRGY  
TGAPVSEEGY  
VQYVGVZY  
VQYVGVZY  
GOPGAPXGZY  
GPPHNGXRAY  
TPPTRRESY  
YTGVSXHF  
AQASAPDAY  
VQYXPF  
VWACV--Y  
PLA-N-HTY  
VVAPIITGY  
XQYTVGYE  
A----ZXEY  
VAGGW---F  
SGAXDRAYZF  
VQGPVGTDF  
FOARXTEY  
XAGFFXEXY  
XQ-----ZY  
FO-----TX  
TP---AZAF  
VVATZNZZX

YMT---F  
APAV---VGY  
PVPNVRXNY  
---TXSX  
YMCNAEEY  
---XRDXY  
SLX-----F  
ALGSZAXMPF  
VGYVDDTQF  
DVEGMSZY  
DVEGMSZY  
YAQHPSAXRF  
-----ZAY  
GLGZTSAEF  
NAXG--RESSE  
TARVXSVEY  
AAFCG---XV  
XLH--ET  
ILGPPGGSVY  
XLGDVNMV  
YVMGXTNANF  
AVVTXZSDF  
AMNPTNTVF  
SQXAAGVDVF  
-VFVSHITF  
XQGHHEMFY  
POGZMA--Y  
HLTGNEATSF  
YMDPSGVSY

SVSXPHAP  
APFTGGNGX  
EHVASSPAL  
HHAPCGVSX  
NHAIIVTSV  
GHZNSVTSTV  
SHZAPCTSV  
FVARFVSX  
HHSDGSVSL  
MCZ-GMPAX  
GHGANNDPAX  
XHSZPAGPAX  
XHVVS--VX  
YHGSZNPEX  
-----M  
EHGXENGH  
AHZAPPTX  
FTACZNPAX  
SHAGAGXVX  
GHXEGPXX  
XHGDDHVX  
YHDXVX  
MAGAWCRX  
FH---XXX  
EH---TVX  
MAX-----VV  
-----PVX  
XHYDRNZX  
-----AXSV  
XHWPNVEX  
-H-----PVE  
XHEVZPXHX  
-HGCPGMPX  
ETPEHAPVX  
MXPGNSAXYX

Fig. 5 continued



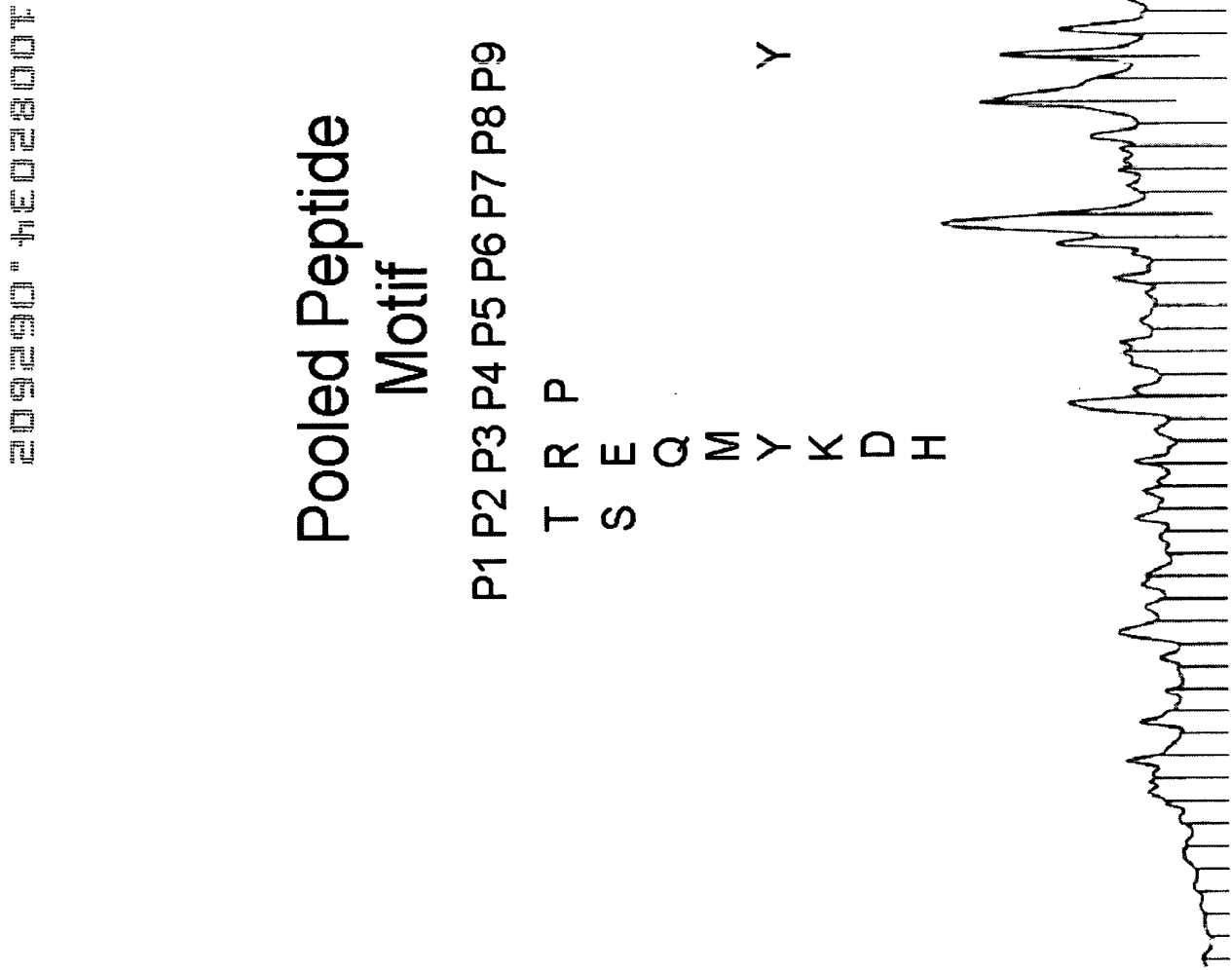


Fig. 6

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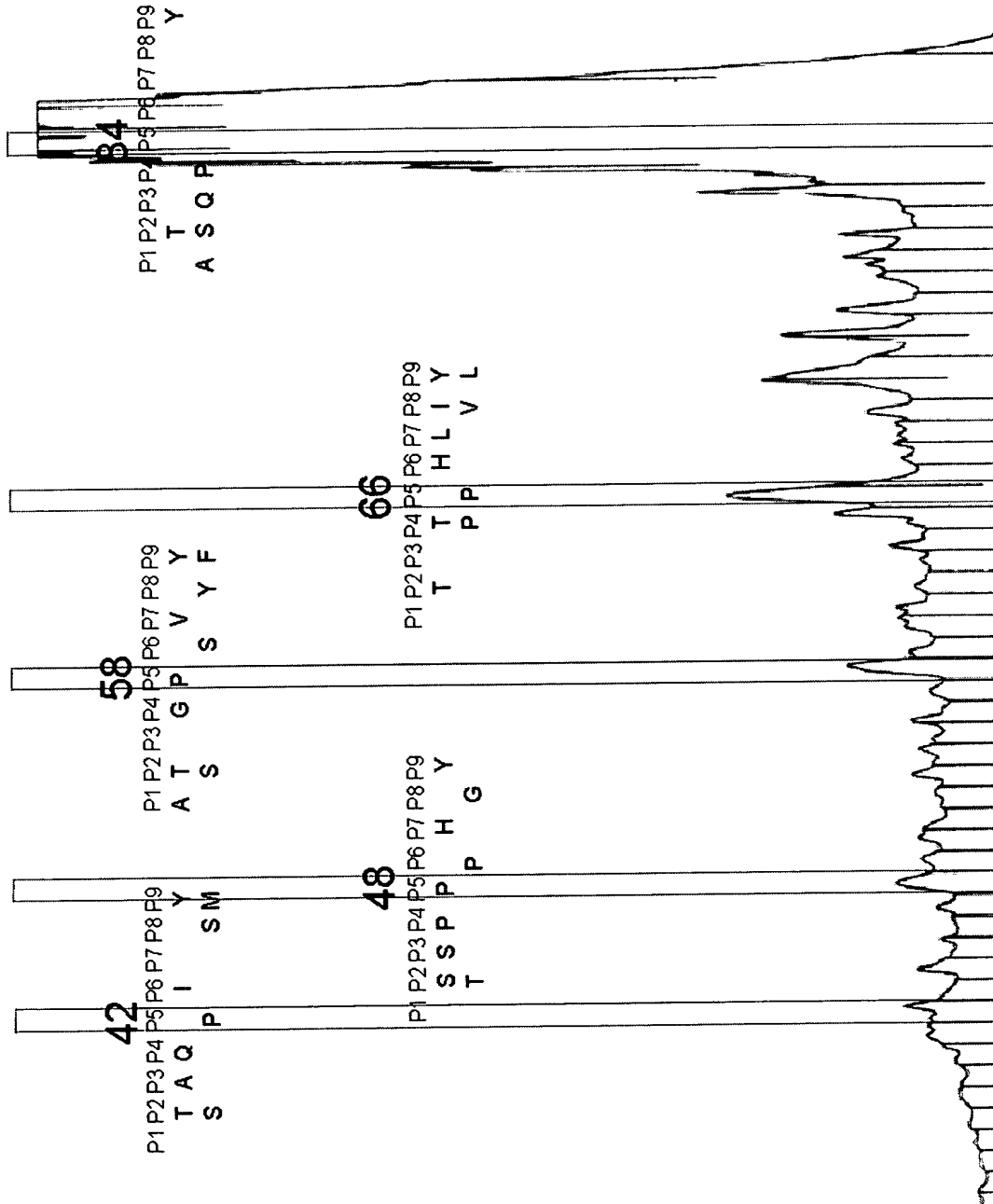


Fig. 7

209290"4E02800T

Narrowing search parameters using fraction motifs:

Ovarian Carcinoma Immunoreactive Antigen				
MNGRADFREP	NAEVRPIPH	IGPDYIPTEE	ERRYFAECND	ESFWFRSYPL
AATSMILTQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSTYVKTCQE
KFKKLENSPL	GEALRSGQAR	RSPPGHHYYQ	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNIKYG
DTWDE				

Scanning with whole-pooled motif revealed 4 putative epitopes.

Ovarian Carcinoma Immunoreactive Antigen				
MNGRADFREP	NAEVRPIPH	IGPDYIPTEE	ERRYFAECND	ESFWFRSYPL
AATSMILTQG	LISKGILSSH	PKYGSIPKLI	LACMGYFAG	KLSTYVKTCQE
KFKKLENSPL	GEALRSGQAR	RSPPGHHYYQ	KSKYDSSVSG	QSSFVTSPAA
QSSFVTSPAA	DNIEMLPHYE	PIPFSSSMNE	SAPTGITDHI	YQGPDNLEE
SPKRKNITYE	ELRNKNRESY	EVSLTQKTDP	SVRPMHERVP	KKEVKVNIKYG
DTWDE				

Scanning with fraction 48 peptide motif revealed 1 putative epitope.

Fig. 8

209230-1E0280DT

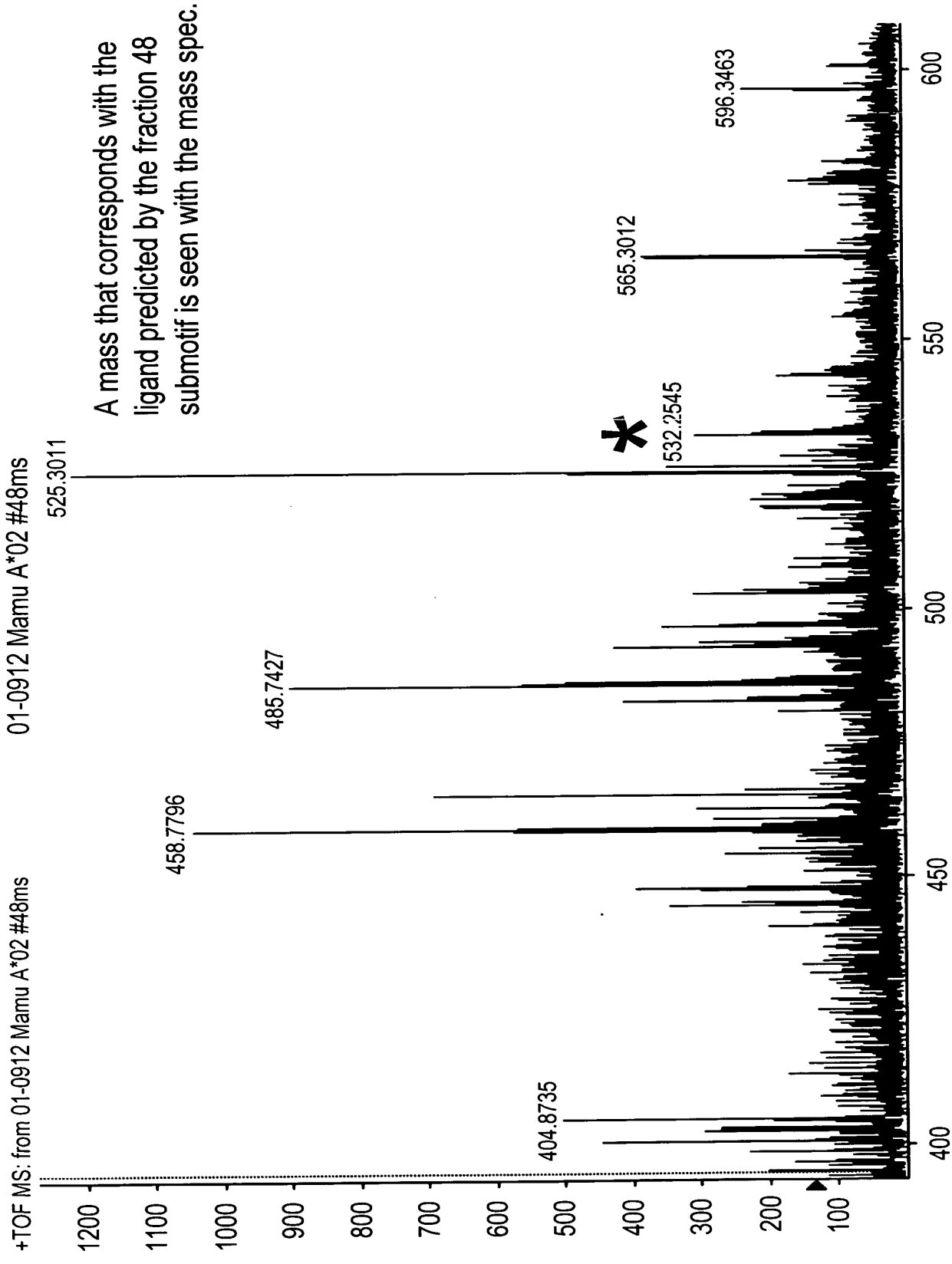


Fig. 9  
m/z, amu

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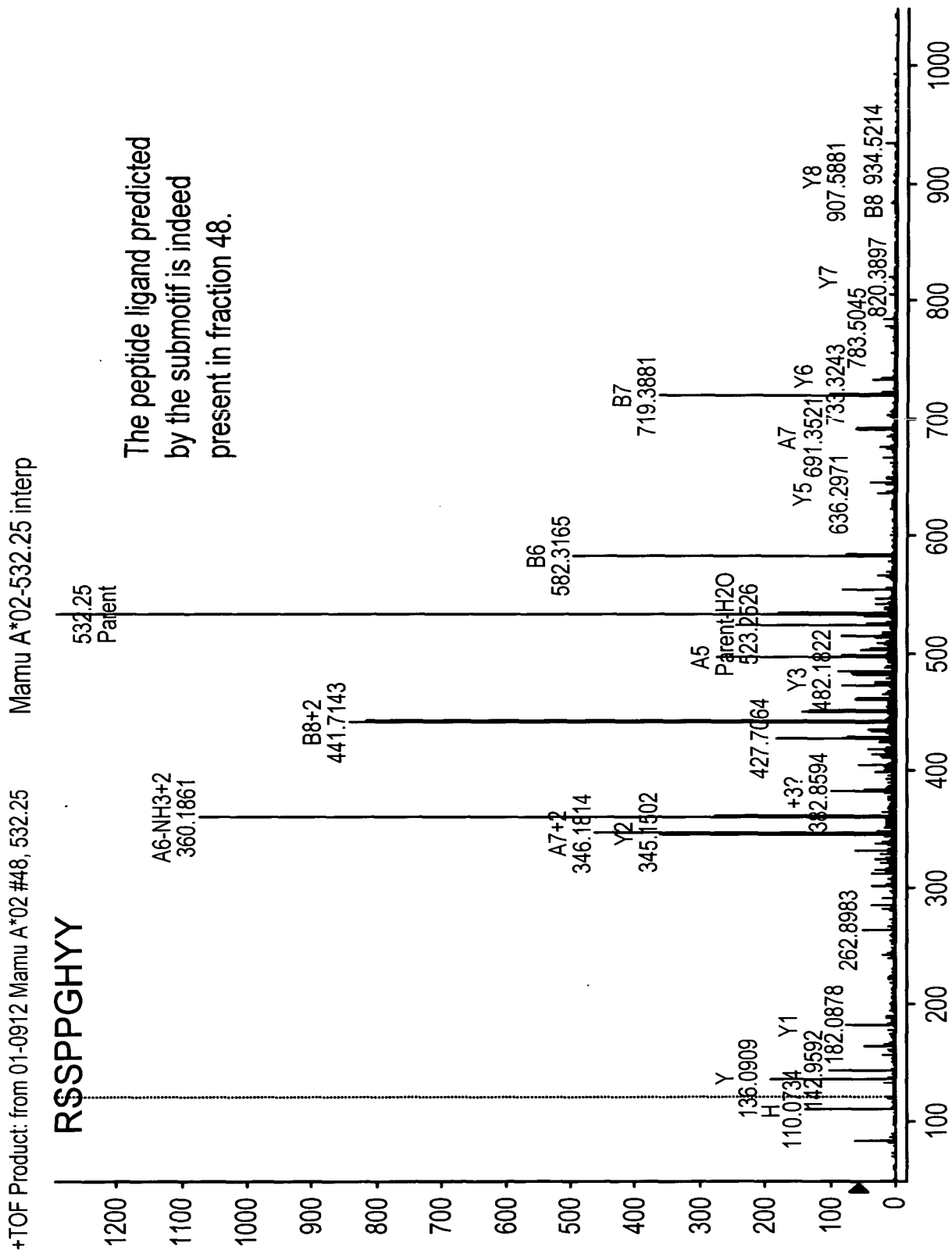


Fig. 10 m/z, amu

Motif Data (Edman sequencing)

	1	%	fold	2	%	fold	3	%	fold	4	%	fold	5	%	fold	6	%	fold	7	%	fold	8	%	fold	9	%
Dominant 3.5 fold increases or more over prior round							F	9.20	11.18																	
							I	7.60	7.01																	
							N	6.20	4.11																	
							M	4.90	10.42																	
Strong 2.5-3.5 fold increase over prior round																										
	K	31.50		R	53.80	2.57	Q	8.20	3.10	P	8.30	2.97													L	7.50
	R	15.50					K	5.40	2.67																	
	S	10.40					L	5.10	3.47																	
Weak 2.0-2.5 fold increase over prior round																										
							A	5.80	2.08				M	4.90	2.28				T	7.70	2.12					
Trace 1.50-2.0 fold increase over prior round				Q	3.60	1.79	P	2.20	1.94	K	11.00	1.63	I	6.50	1.99							Q	11.40	1.93		
										S	6.00	1.68	F	4.20	1.83							N	5.70	1.55		
										V	5.30	1.99	H	1.80	1.67											

Fig. 11

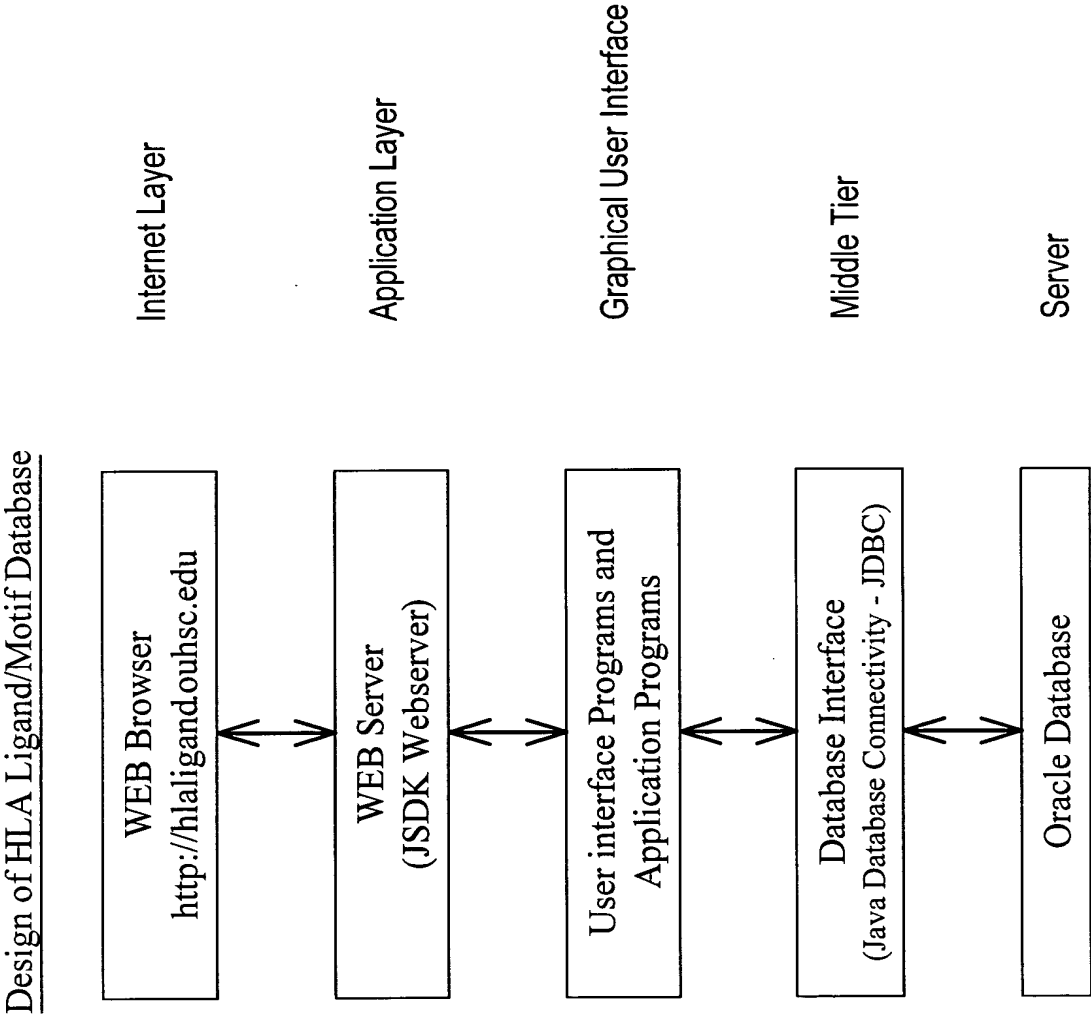


Fig. 12

Entity-Relationship (ER) Diagram for HLA Ligand/Motif Database

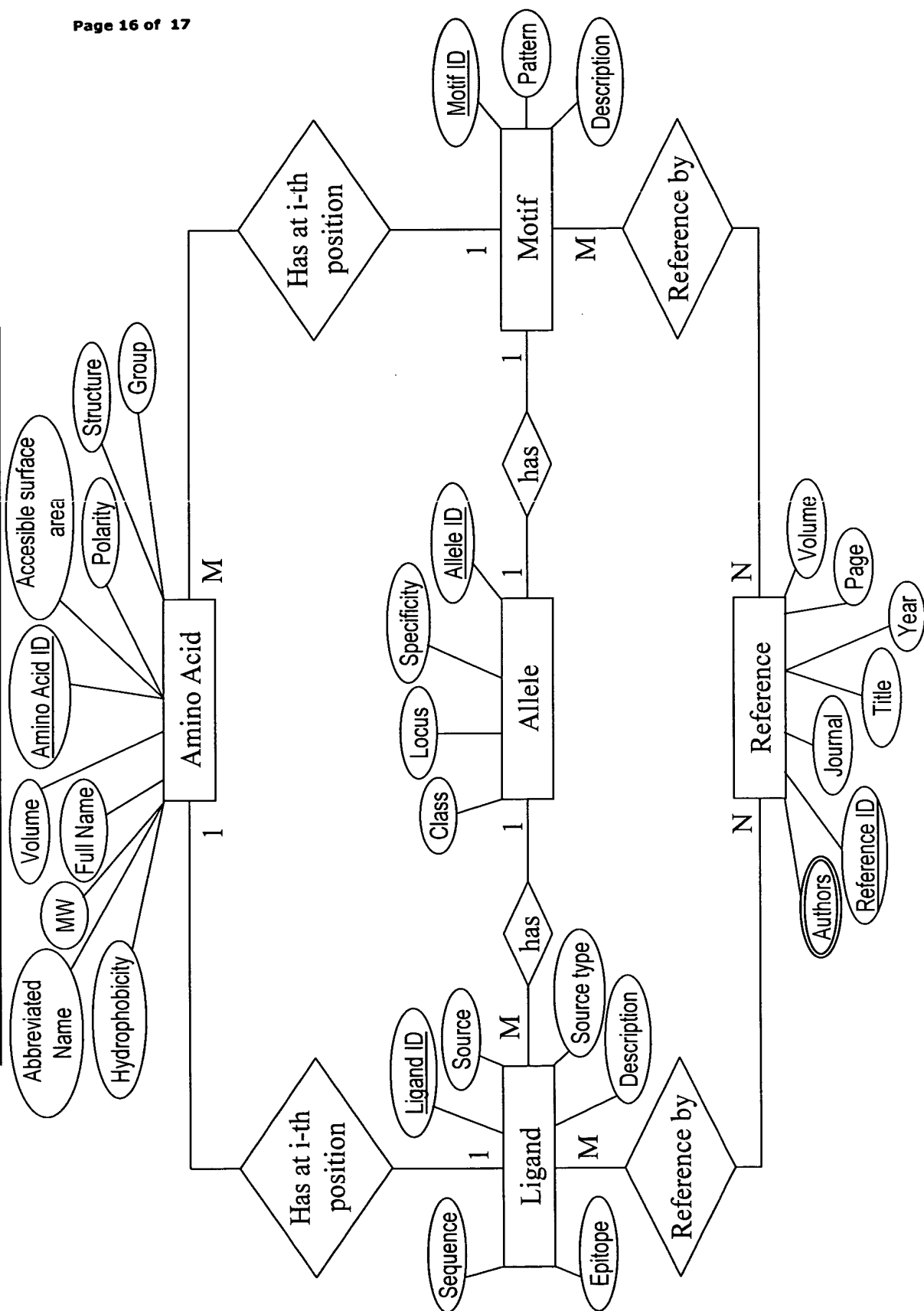


Fig. 13



UML Diagram for HLA Ligand/Motif Database

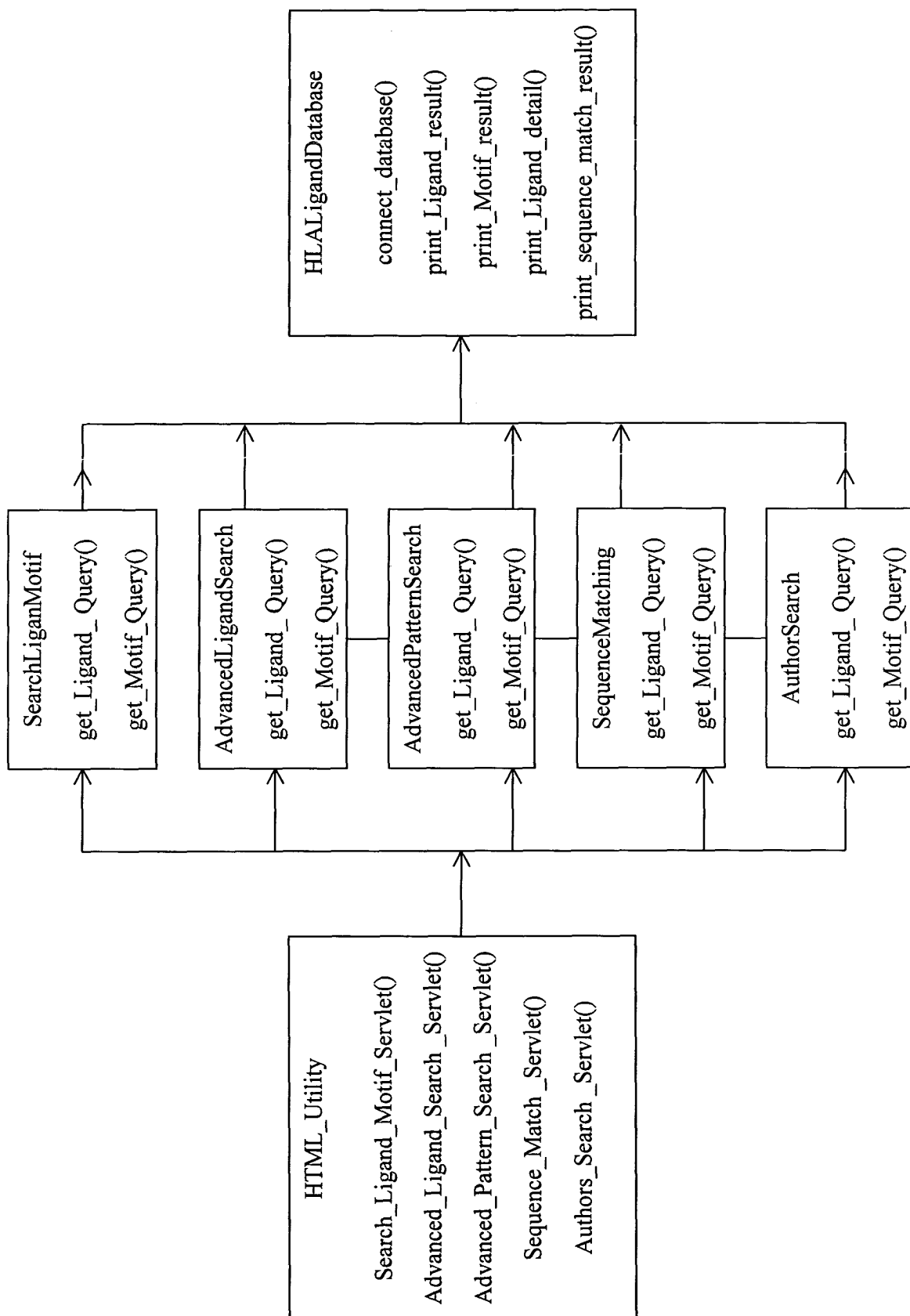


Fig. 14